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14. ABSTRACT

Polycomb group (PcG) proteins, although initially thought to play an exclusive role in development appears to regulate tumorigenesis via regulation of cell proliferation and senescence. Recently it was reported that Bmi-1, a polycomb oncoprotein is overexpressed in breast cancer cells and invasive breast tumors. Bmi-1 overexpression immortalizes breast epithelial cells. Importantly, EZH2, a different polycomb protein was reported to be a marker of aggressive breast cancer and facilitator of neoplastic transformation of immortalized breast epithelial cells. Like any other cancer, breast cancer is a mutlistep process; bypass of cellular senescence and cellular immortalization are considered early steps in the tumorigenesis. Here, we examined the possibility that two different polycomb proteins such as Bmi-1 and EZH2 can together fully transform normal breast epithelial cells. Normal breast epithelial cells (BECs) expressing Bmi-1, CBX-7 and EZH2 alone or in combination were genearated. Cells expressing different combinations of these polycomb proteins were tested for oncogenic properties. Our results indicate that polycomb combinations does not fully transform BECs. However, coexpression of activated H-Ras in Bmi-1 expressing breast cells generated fully transformed phenotype.

15. SUBJECT TERMS
Polycomb group (PcG) proteins, Bmi-1, H-Ras, EZH2, Immortalization, Transformation

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INTRODUCTION

In most cases, breast cancer is a carcinoma arising from the transformation of mammary epithelial cells (reviewed in ref #1). Transformation is a complex multistep process involving several molecular genetic changes (1). It is believed that the first molecular genetic change entails bypass of cellular senescence followed by the immortalization of cells (reviewed in ref #1, 2). After completing a certain number of divisions, normal cells enter a state of irreversible growth arrest and altered function, known as cellular senescence (1, 2). Multiple lines of evidence suggest that cellular senescence constitute a tumor suppressive mechanism (1, 2). In somatic cells, telomerase remains repressed and telomere length keeps shortening at each round of DNA replication. Short telomeres signal cells to stop further proliferation and invoke a permanent growth arrest phenotype known as replicative senescence (1, 2). A variety of genes regulates senescence and proliferation in human cells and cooperates to induce a transformed phenotype (2).

Polycomb group (PcG) proteins, although initially thought to play an exclusive role in development appears to regulate tumorigenesis via regulation of cell proliferation and senescence (3). Recently, it was found that Bmi-1, a polycomb oncoprotein is overexpressed in breast cancer cells and that it can immortalize breast epithelial cells (4). Similarly, it was reported that exogenous overexpression of another polycomb protein CBX7 bypasses senescence and extend the replicative life span of human prostate epithelial cells (5). EZH2, a different polycomb protein was reported to be a marker of aggressive breast cancer and facilitator of neoplastic transformation of immortalized breast epithelial cells (6). Bmi-1 was also reported to be required for stem cell renewal and was speculated to be an important determinant of breast cancer stem cell. Thus, polycomb proteins can affect early as well as late steps in breast cancer tumorigenesis. Here, we examined the possibility that two different polycomb proteins can fully immortalize and transform normal human breast cells, also referred as human mammary epithelial cells (HMEC).

BODY:

Generation of HMEC cell lines overexpressing different polycomb combinations:

We have previously generated Bmi-1 overexpressing HMEC strain 76N (4). Here, we generated 76N cells that express combination of Bmi-1 and CBX7, and Bmi-1 and EZH2. CBX7 and EZH2 cDNA were PCR amplified with a Myc tag, cloned in a retrovirus vector pLPC. Retrovirus expressing Myc-CBX7 and Myc-EZH2 were generated by trasfection into a packaging cell line tsa54 as described (4). 76NBmi-1 cells were infected with Myc-CBX7 or Myc-EZH2. Expression of CBX7 and EZH2 was verified using Myc antibody (Figure 1). Control 76N cells expressing a single polycomb either CBX7 or EZH2 were also generated. Cells expressing single polycomb protein such as Bmi-1, CBX-7 and EZH2 were continually passed in culture to study the immortalization potential of cells. Consistent with our earlier study only Bmi-1 overexpression led to immortalization (4). Myc-CBX7 and Myc-EZH2 alone failed to immortalize HMECs. Since Myc-CBX7 and Myc-EZH2 expressing cells were not immortal, telomerase assay was not performed.

A combination of polycomb proteins (Bmi-1+CBX7 and Bmi-1+EZH2) were also overexpressed in another widely used cell line MCF10A, which is an immortal but non-transformed HMEC cell line. MCF10A cells were sequentially infected with Bmi-1 overexpressing retrovirus followed by retrovirus expressing either EZH2 or CBX7. H-Ras is known to cooperate with cellular and viral oncogenes to induce transformed phenotype in HMECs. Hence, we also overexpressed activated H-Ras in 76N-Bmi-1 cells to examine the

possibility of polycomb cooperating with H-Ras to transform HMEC. As a control, H-Ras was also overexpressed in 76N cells immortalized with hTERT (telomerase catalytic subunit).

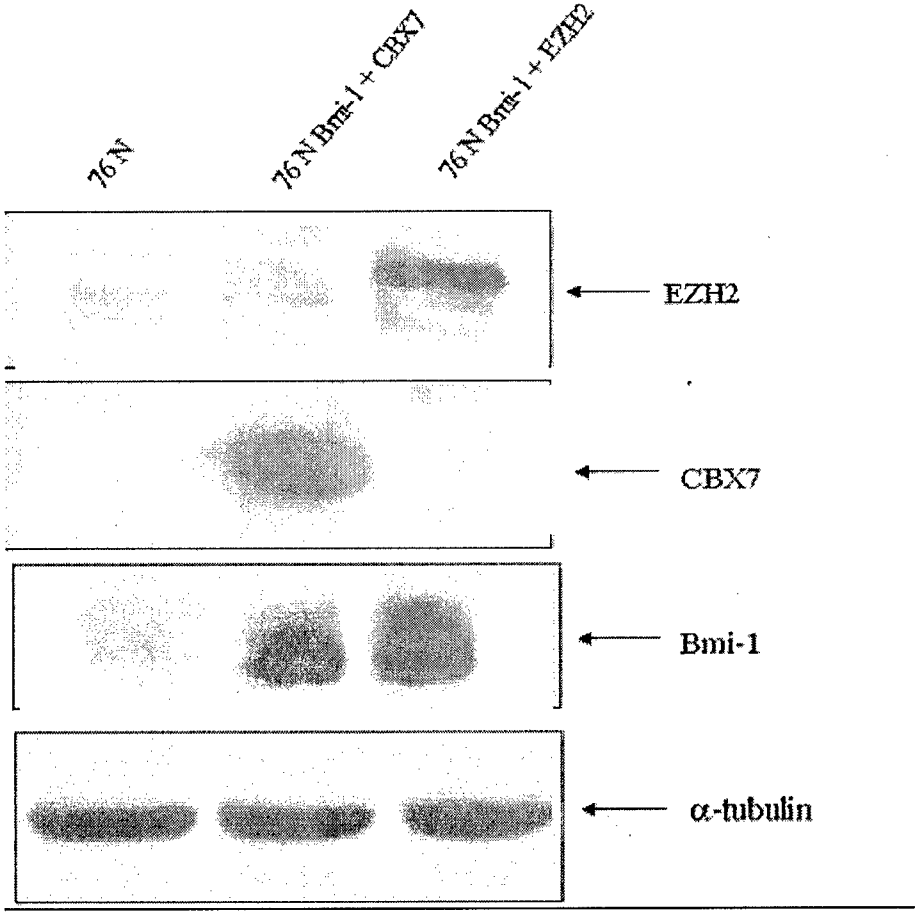

Figure 1: Western blot analysis of various polycomb proteins in HMEC 76N strain. Expression of Bmi-1 and α-tubulin (loading control) was determined using respective antibodies as described (4). Expression of EZH2 and CBX7 was determined using Myc antibody.

Transformed phenotype of polycomb expressing HMECs:

To determine the transformation potential of different combinations of polycomb 76N cells and MCF10A cells expressing polycomb proteins were plated in soft-agar as described (6). MCF7 cells, which are highly transformed and MCF10A, which are non-transformed were used as a positive and negative control respectively. Colony formation in soft agar was observed and colonies were photographed.10 days after plating. The results showed that polycomb combinations studied here did not induce transformed phenotype (Figure 2). Colonies were observed only in MCF7, and 76N cells expressing combination of Bmi-1 and H-Ras. 76N or MCF10A cells expressing Bmi-1+CBX7 or Bmi-1+EZH2 failed to form colonies in soft-agar indicating expression of these two polycombs is not sufficient to induce full transformed phenotype in HMECs. These data are summarized in Table 1. Since cells expressing polycomb combinations failed to grow in soft agar, we did not study invasive potential of these cells.

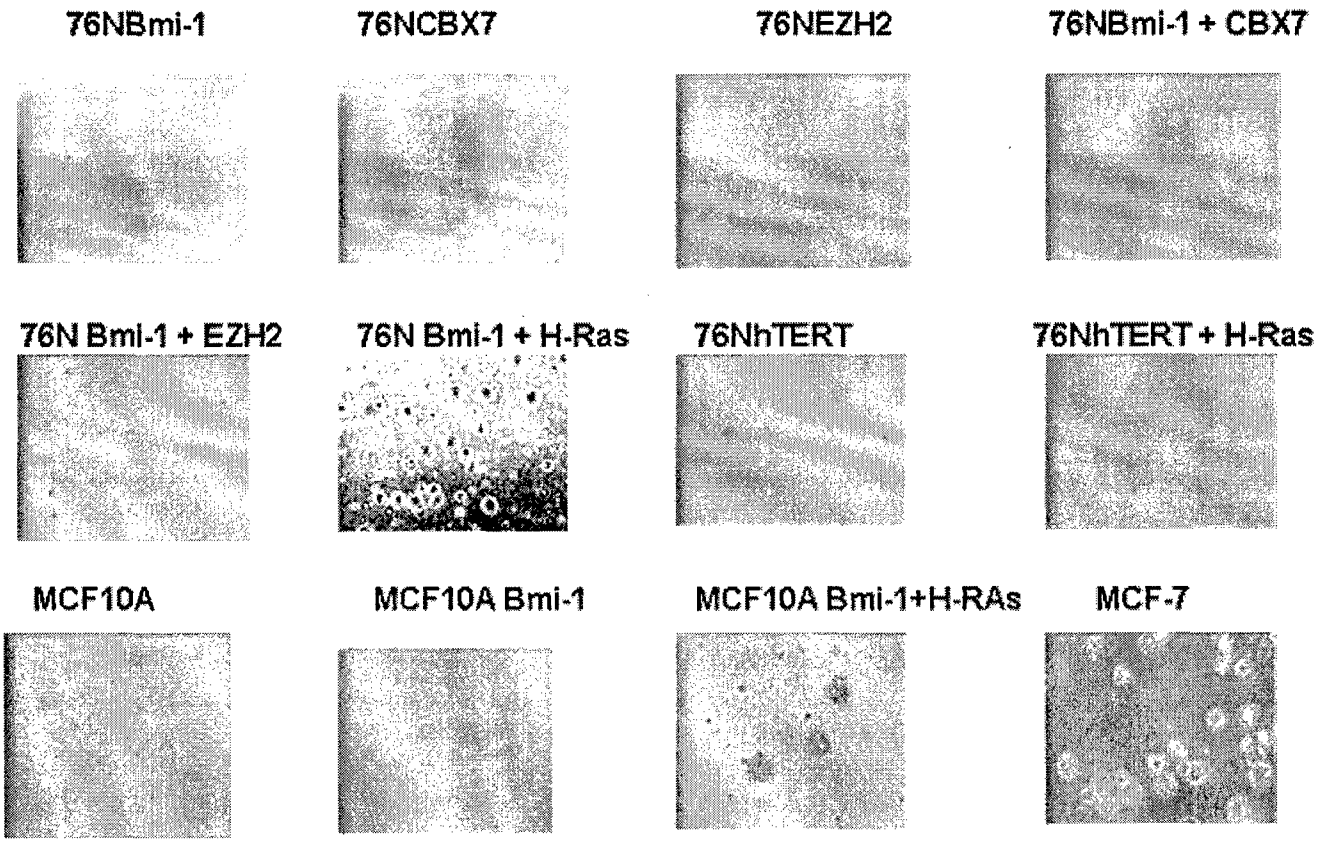

Figure 2: Anchorage independent growth of polycomb proteins expressing HMECs in soft-agar.

Table 1

Cell Type	Soft Agar Colony Formation
76N-Bmi-1	-
76N-CBX7	-
76N-EZH2	-
76N-Bmi-1+CBX7	-
76N-Bmi-1+EZH2	-
76NBmi-1+H-RAS	+++
76NhTERT+H-RAS	-

MCF10A-Bmi-1	-
MCF10A-Bmi-1+H-Ras	++
MCF10A-CBX7	-
MCF10A-EZH2	-
MCF10A-Bmi-1+CBX7	-
MCF10A-Bmi-1+EZH2	-
MCF7	+++
76NhTERT	-

KEY RESEARCH ACCOMPLISHMENTS:

1. We have generated various strains HMEC expressing single polycomb protein or combination of these proteins.
2. We have found that polycomb combination alone may not be sufficient for full transformation of HMECs (breast epithelial cells).

REPORTABLE OUTCOMES:

None

CONCLUSIONS:

In this study, we examined cooperation between two different polycomb proteins, in particular Bmi-1+EZH2, and Bmi-1+CBX7 in human breast cell transformation. Based on data presented here these combinations did not induce transformed phenotype suggesting polycombs may not cooperate to transform HMECs. However, it remains possible that different combinations of polycombs may transform HMECs or polycomb combination may need inactivation of pRb and/or p53 tumor suppressors to transform HMECs. We also note that in our studies we used Myc tagged CBX7 and EZH2. It is known that sometime tag can interfere with biological function of a particular protein. We are currently exploring these various possibilities. On the other hand, Bmi-1 strongly cooperated with activated H-Ras to transform HMECs suggesting polycomb may cooperate with H-Ras or H-Ras activated pathways during breast tumorigensis.

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